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Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa

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HIGHLIGHTS

- Efavirenz, nevirapine and carbamazepine were detected in all Hartbeespoort Dam water samples.
- Nevirapine and carbamazepine were the most abundant EPs in Hartbeespoort Dam groundwater.
- Efavirenz was found in the highest concentration in the Umngeni River estuary composite water sample.
- Nevirapine was found at the highest concentrations the Umngeni River estuary sediments.
- Hartbeespoort Dam fish muscle (carp and catfish) did not significantly bioaccumulate EPs.

GRAPHICAL ABSTRACT

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ABSTRACT

A quantitative assessment of pollutants of emerging concern in the Hartbeespoort Dam catchment area was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to establish the occurrence, source and distribution of 15 environmental pollutants, including 10 pharmaceuticals, 1 pesticide and 4 steroid hormones. Seasonal sampling was conducted in the Hartbeespoort Lake using sub-surface grab sampling to determine the lake's ecological status and obtain data for establishment of progressive operational monitoring. The Jukskei River, which lies upstream of the Hartbeespoort Dam, was sampled in the winter season. Five year old carp (*Cyprinus carpio*) and catfish (*Clarias gariepinus*) were also sampled from the Hartbeespoort Dam to study bioaccumulation in biota as well as to estimate risk associated with fish consumption. In the Jukskei River, the main source of 11 emerging pollutants (EPs) was identified as raw sewage overflow, with the highest $\sum$11 EP concentration of 593 ng L^{-1} being recorded at the Midrand point and the lowest $\sum$11 EP concentration of 164 ng L^{-1} at the N14 site located 1 km downstream of a large wastewater treatment plant. The Jukskei River was found to be the largest contributor of the emerging contaminants detected in the Hartbeespoort Dam. In the Hartbeespoort Dam EP concentrations were generally in the order efavirenz > nevirapine > carbamazepine > methocarbamol > bromacil > venlafaxine. Water and sediment were sampled from the uMngeni River estuary within 24 h after large volumes of an assortment of pharmaceutical waste had been discovered to be washed into the river estuary after flash rainfall on 18 May 2016. Analytical results revealed high levels of some emerging pollutants in sediment samples, up to 81 ng g^{-1} for nevirapine and 4 ng g^{-1} for etilefrine HCL. This study shows that

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efavirenz, nevirapine, carbamazepine, methocarbamol, bromacil and venlafaxine are contaminants that require operational monitoring in South African urban waters.

1. Introduction

An emerging contaminant is an unregulated compound that is not included in regular environmental monitoring programs and for which there is only limited knowledge about its toxicity and behavior in the environment, but has the potential to cause adverse effects to the ecosystem (López-Doval et al., 2017; Pal et al., 2010; Sauvé and Desrosiers, 2014). Emerging contaminants are seldom monitored in South African waters, as the significance of monitoring them is poorly understood. Therefore, the focus is largely on legacy contaminants and toxic persistent organic pollutants. Emerging pollutants include active pharmaceutical ingredients (APIs) or biologically active compounds, some of which have been recently formulated, but widely used due to their efficacy (Metcalfe et al., 2014; Maruya et al., 2014; Pal et al., 2010). The analytical power of instrumentation and techniques to screen and quantify emerging contaminants has significantly improved over the past two decades, enabling the discovery of more emerging pollutants (Raldúa et al., 2011; Sauvé and Desrosiers, 2014; Valavanidis et al., 2014). Due to the large number of APIs present in water bodies impacted by wastewater treatment plant effluents as well as hospital and manufacturing waste, prioritising which API's to monitor may prove to be a challenge (Caldwell et al., 2014; Besse and Garric, 2008). API's are more likely to be found in the water fraction than in the sediment and air fractions due to their high polarity and low volatilities (Crane et al., 2006).

Ideally, predicted effect concentrations provide an acceptable way of identifying pollutants of high priority for monitoring in the environment, along with exposure, persistence, bioaccumulation and toxicity (EPBT) data (Ebele et al., 2017; Fick et al., 2010; Mansour et al., 2016). However much of this information is scanty for emerging pollutants (Besse and Garric, 2008). A constant exposure to API contaminants in aquatic reservoirs may have effects on non-target organisms such as altering homeostasis of exposed aquatic organisms, acute toxicity and development of pharmaceutical drug resistance in microorganisms due to chronic exposure at low concentrations (Agunbiade and Moodley, 2014; Ebele et al., 2017; Farré et al., 2008; Lei et al., 2015; Maruya et al., 2014; Pereira et al., 2015). As traces of antibiotics in water have been found to induce antibacterial resistance, the threat of antiretroviral resistance due to ubiquitous antiretroviral (ARV) drugs and metabolites in South African waters may be worth investigating (Sauvé and Desrosiers, 2014). A study by Swanepoel et al. (2015) has detected ARV drugs in groundwater as well as tap water sampled from the Gauteng Province of South Africa and ARV drugs in groundwater have been previously reported by K'Oreje et al. (2016) in Kenya.

The effects of pharmaceuticals on the ecosystem may not be noticeable without considerable scrutiny, however, there is growing evidence of adverse effects of some pharmaceuticals on exposed aquatic animals (Klatte et al., 2017). With the exception of 17-β-estradiol and 17-α-estradiol, the majority of pharmaceuticals monitored in the environment do not appear to cause significant endocrine disruption or toxic effects in aquatic animals as they are usually below the therapeutic concentrations (Brumovský et al., 2016; Caldwell et al., 2014; Crane et al., 2006; Jálová et al., 2013; Schoenfuss et al., 2016). However, the effect of a cocktail of pharmaceutical contaminants in water may produce a significant adverse effect compared to a single pharmaceutical contaminant (Schoenfuss et al., 2016). Antidepressants and central nervous system drugs including venlafaxine and methocarbamol may be linked to neurobehavioral disorders in aquatic animals with reports of diminished predator evasive behavior and less aggressive nest defense in exposed fish (K'Oreje et al., 2012; Schoenfuss et al., 2016). Morphological examinations of fish exposed to pharmaceuticals show enlarged livers, an observation that may be linked to the fact that most ARV drugs cause variable degrees of liver damage and hepatotoxicity in humans (Schoenfuss et al., 2016).

The Hartbeespoort Dam is situated downstream of the largest wastewater treatment plant in Johannesburg, Gauteng Province and is one of the major agricultural dams in South Africa. The Madibeng municipality draws water from the Hartbeespoort Lake for treatment and supply to the nearby Hartbeespoort Dam community, however it has also been implicated for contaminating the Hartbeespoort Dam with sewage overflow. Biologically active pharmaceuticals originating from the Northern wastewater treatment works (WWTW) and raw sewage spills contaminating the Jukskei River may potentially contaminate aquatic life and irrigated crops downstream as most wastewater treatment plants are not designed to eliminate active pharmaceutical ingredients and other organic compounds (Barbosa et al., 2016). Operational and investigative monitoring of emerging contaminants in water bodies may provide useful information to water resource managers, particularly in times of episodic contamination. An episodic pollution event recorded on 18 May 2016 caused large quantities of improperly disposed medical waste to be deposited on the uMngeni River estuary, located north of Durban city centre in KwaZulu-Natal province, as well as the adjacent beach after sudden flash rainfall. As a result, the beach was closed to the public for massive cleanup campaigns which took >3 days to complete. In spite of the pollution incident, the beach was re-opened to the public within 24 h after the incident was discovered, before any scientific tests could be conducted on the water quality. During the estuary cleanup numerous pharmaceutical bottles containing mainly expired medicines were found on the uMngeni estuary and beach front, including multivitamins as well as ARV and hypertension drugs. The source of the pharmaceutical waste was never identified, hence accountability as stipulated in the South African National Environmental Management Act 107 of 1998 (which states that the waste holder should take steps to ensure that waste does not endanger health or the environment) was not implemented because investigations were inconclusive. Despite South Africa having formidable environmental laws, environmental compliance monitoring and enforcement has often been viewed as being ineffective (DEA, 2012).

The aim of this research was to study the seasonal variation of selected emerging pollutants (EPs) and to assess their occurrence and transport in the Hartbeespoort Dam catchment, which is impacted by a large wastewater treatment plant and numerous informal settlements which contribute raw sewage into the catchment. It was also aimed to assess the impact of an episodic event of improperly disposed pharmaceutical waste washing up on the uMngeni River estuary by analysis of the 15 EPs in water and sediments sampled within 24 h after the pollution incident was discovered.

2. Materials and methods

2.1. Study area and sampling

Five sites located primarily around the major inlets and outlet of the Hartbeespoort Dam (Fig. 1) were sampled to determine the point with the greatest influence on the Hartbeespoort Dam pollution. Another 5 points located at pollution hotspots upstream of the Hartbeespoort Dam, in the Jukskei River (Fig. 1) were selected for the study. The GPS coordinates are listed in Supporting Information (SI) Table S1. The Jukskei River and the Hartbeespoort Dam are effluent dominated waters (Wimberly and Coleman, 1993). The Jukskei River passes through the

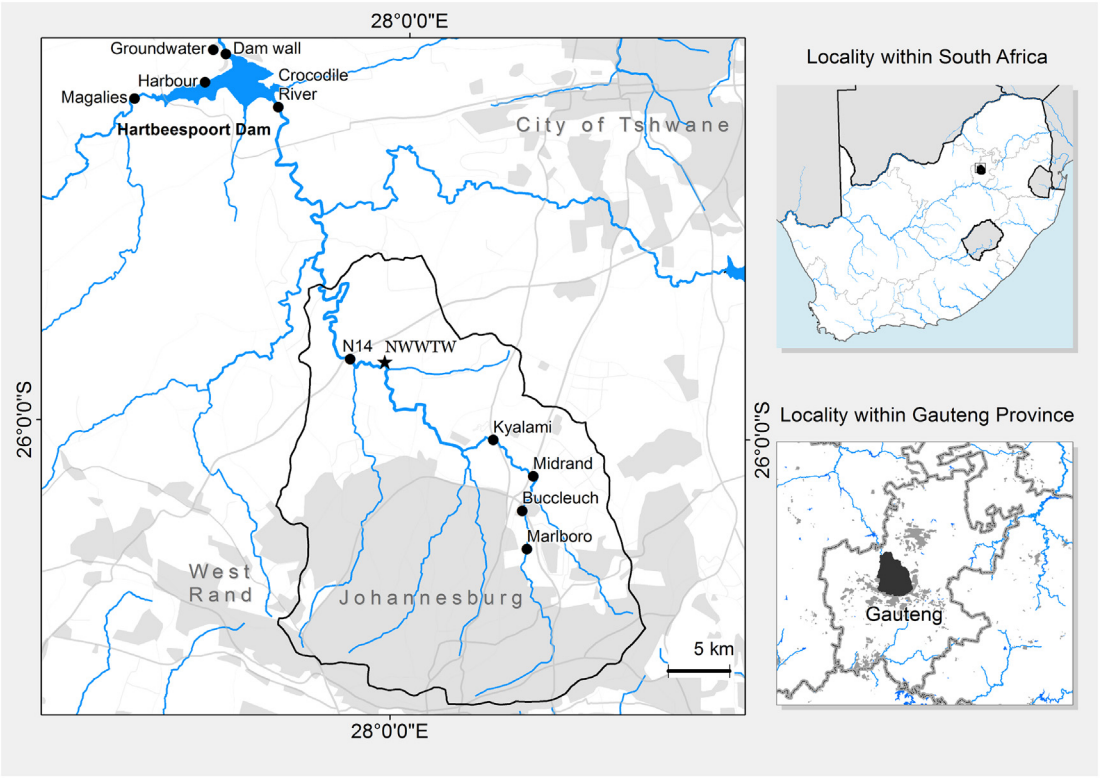


Fig. 1. Sampling area - Hartbeespoort Dam catchment, Gauteng province, South Africa. NWWTW = Northern Wastewater Treatment Works (Municipal sewage treatment plant). The Jukskei River catchment is shown in black border.

largest and most densely populated and industrialised region in South Africa. The Jukskei River sampling points of Marlboro, Buccleuch, Midrand and Kyalami (Fig. 1) are located in areas heavily impacted by raw sewage where numerous informal settlements with no municipal sewage facilities are sources of organic pollutants, particularly upstream of the Marlboro site as well as downstream of the Midrand site. Other point sources include municipal and industrial waste waters as well as the Northern WWTW which is the largest wastewater treatment plant in Johannesburg, serving 1.6 million people with a sewage capacity of 360 million L day^{-1}. The Northern WWTW is located adjacent to the Jukskei River, upstream of the N14 site where the effluent is discharged (Amdany et al., 2014). The sites were selected to determine the effect of the wastewater treatment plant as well as untreated sewage continuously contaminating the Jukskei River between the Marlboro and Midrand sites. At the Hartbeespoort Dam, the groundwater site is situated in close proximity to the Dam wall point (1.1 km away) and the water is used for drinking purposes by the surrounding community and government offices.

The uMngeni River estuary lies downstream of an expanse of municipal, rural, industrial and agricultural lands (Matongo et al., 2015). A composite sub-surface water sample was sampled across the uMngeni River estuary from a boat, along site U2 (Fig. 2) using sub-surface (2–3 cm below the water surface) grab sampling, filling a clean 4 L amber glass bottle. Sediment samples were taken at sites U1 to U4 (Fig. 2) to assess the risk and concentrations of medical waste and expired pharmaceuticals (suspected to have been dumped along the uMngeni River) washed downstream by flash rainfall. All samples were taken in a random manner within a 2 m radius and placed in a cooler box with ice packs before transporting by air to the laboratory cold room maintained at 5 °C. Site U4 is located on the mouth of the uMngeni River estuary (Fig. 2). Sites U1, U2, U3 are located 700 m, 250 m and 120 m from the mouth of the estuary (U4) respectively. The GPS coordinates are listed in SI Table S2.

Sampling in the Hartbeespoort Dam was conducted between November 2014 and September 2015 (SI Table S3), over 4 seasons and the Jukskei River was sampled in May 2015 (winter season). The uMngeni River estuary lies in the eThekwini municipal area (Fig. 2) and was sampled on 19 May 2016. Sediment samples (top 0–5 cm) were sampled using a Van Veen grab sampler and collected in a clean 500 mL glass jar. Water samples were sampled using sub-surface (2–3 cm below the water surface) grab sampling and a bailer for the Hartbeespoort Dam wall point at 5 m and 30 m depths. The water samples were collected in a clean 4 L amber glass bottle. All samples were taken in a random manner within a 2 m radius from within a boat and placed in a cooler box with ice packs before being transported to the laboratory where they were stored at 5 °C prior to analysis. Three 5 year old catfish (*Clarias gariepinus*) and three 5 year old carp (*Cyprinus carpio*) were caught on 14 August 2015 from the Hartbeespoort Dam using mobile nets and immediately frozen at the Hartbeespoort Dam site before transporting to the laboratory freezer in a cooler box packed with ice. The age of the catfish was estimated by using length-weight ratios and the age of the carp was estimated using a combination of length-weight ratios and scale annuls, utilizing scales taken from between the lateral line and pectoral fin. The seasons (SI Table S3) and climate of the sampling area has been described in detail elsewhere (Rimayi et al., 2018).

2.2. Chemicals

All internal and native standards had a purity ≥ 96% (Tables 1 & 2) and were supplied by Dr. Ehrenstorfer and Toronto Research Chemicals (Industrial Analytical Johannesburg, South Africa). All solvents had a purity > 99.9%. Ultrapure Milli-Q water used in all preparation work was produced by a Millipore Advantage system (Merck, Johannesburg, South Africa) with a TOC < 3 mg L^{-1}.

2.3. Sample analysis

2.3.1. Water sample analysis

1 L water samples for LC-MS/MS analysis were extracted using 200 mg Agilent Bond Elut Plexa (Stryrene divinyl benzyl) solid phase

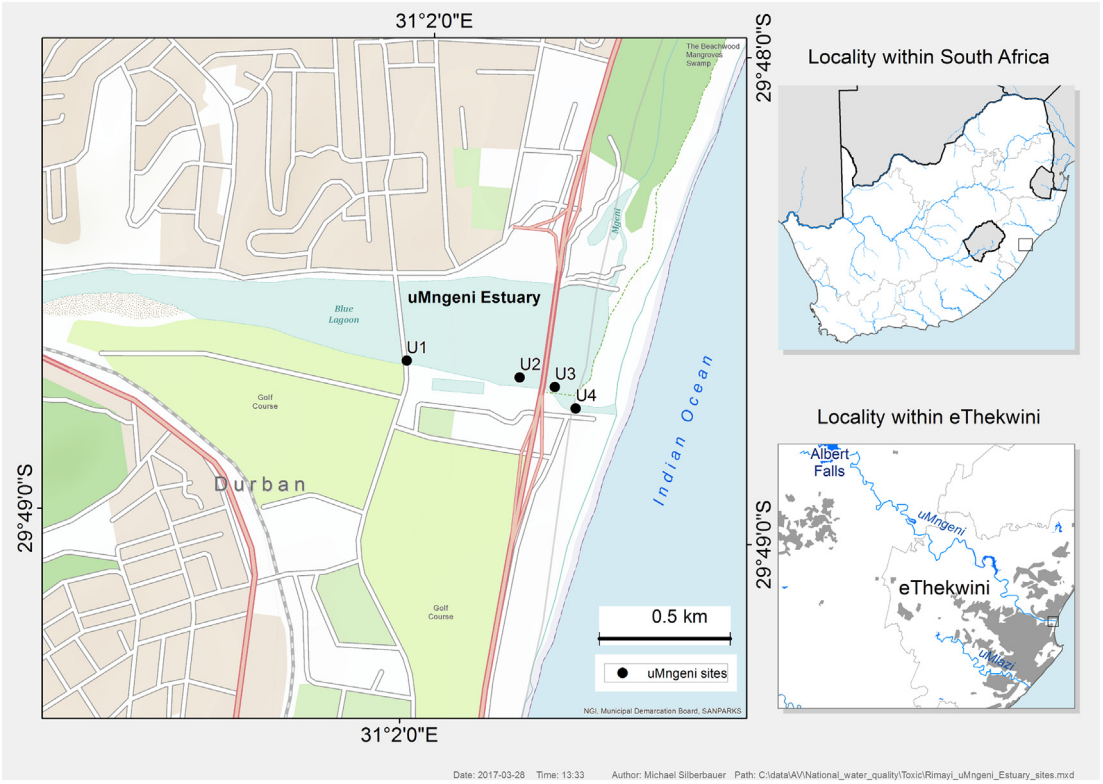


Fig. 2. Sampling area - uMngeni River estuary.

extraction cartridges (Chemetrix, Johannesburg, South Africa) according to the method described by Rimayi et al. (2018). The sample extracts were reconstituted with 200 μL of 10% methanol (Milli-Q water: Methanol, 90:10 v/v).

2.3.2. Fish analysis

The modified Quick Easy Cheap Efficient Rugged Safe (QuEChERS) analytical procedure used for the analysis of the 5 year old free range carp and catfish muscle has been described elsewhere (Rimayi et al., 2018).

2.3.3. Sediment analysis

Sediments were freeze dried using a Christ Alpha 1–4 LD plus freeze dryer (Lasec, Johannesburg, South Africa), granulated with a mortar and pestle and sieved through a 2 mm sieve before transporting to The Netherlands under frozen conditions where extraction was performed according to the EPA method 3545A using dichloromethane and acetone 1:1 (v/v) under conditions described by Rimayi et al. (2016).

2.3.4. Particle size distribution

The particle size distribution was measured using an automated Retsch AS200 filterflow instrument set at aptitude 60 for 2 min, using ISO 3310-1 certified sieves with mesh sizes of 2000, 424, 100, 50 and 25 μm. The sieved fractions were weighed using a calibrated Precisa 180A, 5 decimal place balance. Stones and debris > 2 mm were removed using a 2 mm mesh size sieve.

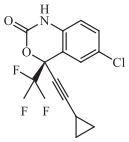
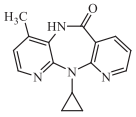
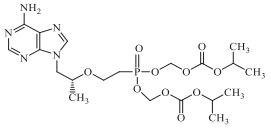
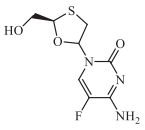
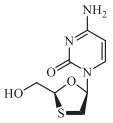
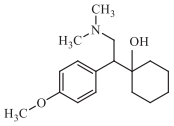
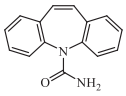
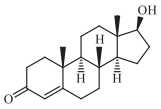
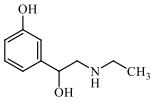
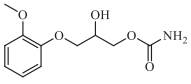
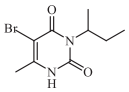
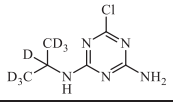
2.3.5. Total organic carbon

A modified Walkley-Black method was used to calculate the total organic carbon (TOC) in sediments according to the method described by Rimayi et al. (2016). The sediment samples were weighed into a digestion tube before adding 8 mL of 1 N $K_2Cr_2O_7$ (Minema, Johannesburg, South Africa) and digesting the mixture for 30 min at 150 °C using a digestion block. After cooling, the digestant was transferred to a conical flask and rinsed with 25 mL deionised water before adding 2 mL H_3PO_4 to eliminate interfering Fe^{3+} ions. Eight drops of 0.2% barium diphenylamine-4-sulfonate (Sigma-Aldrich, Missouri, USA) colour indicator solution were added before back titrating the residual $K_2Cr_2O_7$ to a green endpoint with 1 M ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2 * 6H_2O$) solution (BDH Merck Limited, Poole, England).

2.3.6. LC-MS/MS method

An LC-ESI-MS/MS (1200 series LC system, 6410 triple quadruple MS; Agilent Technologies, Amstelveen, The Netherlands) instrument utilizing a 100 × 2.1 mm Kinetex 2.6 μm Biphenyl liquid chromatography (LC) column (100 Å) maintained at 25 °C was used. The mobile phase for compounds not derivatised (Table 1) consisted of Solvent A: 5 mM ammonium formate in Milli-Q water (pH 4, formic acid) and Solvent B: 1.5% formic acid in methanol. Solvent A gradient was held at 100% for 2 min before increasing solvent B linearly to 20% (hold 8 min), increasing solvent B linearly to 95% (hold 5 min) and returning to 100% solvent A at 15.5 min (hold 14.9 min). A 10 μL injection volume was used with a constant flow of 0.3 mL min^{-1}. Tandem mass spectrometry was (MS/MS) was coupled to an Electron Spray Ionization (ESI) source in positive mode with source spray voltage 4 kV, transfer capillary temperature 350 °C, capillary voltage +4000 V, nitrogen drying gas flow 9 mL min^{-1} and nebulizer pressure 40 psi. For dansylated compounds (Table 2), solvent A consisted of 1% formic acid in Milli-Q water and solvent B consisted of 1.5% formic acid in methanol. A dansyl chloride derivatisation procedure was utilized to achieve a calibration range of 8 to 0.1 ng L^{-1} for 17-β estradiol, 17-α estradiol, D4 estrone, *p*-chloroaniline and benzestrol. The derivatisation procedure employed is described elsewhere (Nelson et al., 2004) and yielded monodansylated 17-β estradiol, 17-α estradiol and *p*-chloroaniline as well as bidansylated benzestrol. A calibration range of 200 to 0.2 ng L^{-1} was used for the underivatised compounds. Data was acquired in dynamic MRM mode and analysed on a computer with MassHunter quantitative analysis software (Palo Alto, USA).

Table 1
Target database and internal standards.

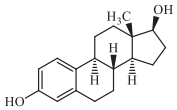
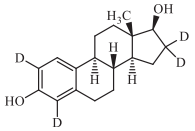
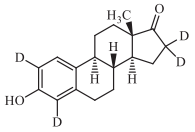
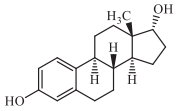
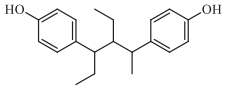
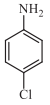
Compound	Structure	Purity (%)	Compound type	Quantification ion	Confirmation ion 1 (Collision Energy)	Confirmation ion 2 (Collision Energy)	Therapeutic dose side effects on humans[a]
Efavirenz CAS# 154598-52-4 $C_{14}H_9ClF_3NO_2$ logP: 4.46		100	NNRTI	53.2 (60)	167.5 (20)		Moderate to severe liver problems
Nevirapine CAS# 129618-40-2 $C_{15}H_{14}N_4O$ logP: 2.49		100	NNRTI	78.2 (68)	51.1 (112)		Hepatotoxicity causing fatal liver problems, life threatening skin reaction
Tenofovir disoproxil CAS# 202138-50-9 $C_{19}H_{30}N_5O_{10}P$ logP: 2.65		98	NRTI	176.5 (56)	159.4 (64)	136.5 (72)	Severe liver problems, severe lactic acidosis
Emtricitabine (mikromol) CAS# 143491-57-0 $C_8H_{10}FN_3O_3S$ logP: −0.90		100	NRTI	130.3 (8)	85.3 (56)	58.3 (72)	Lactic acidosis, liver damage, skin reaction
Lamivudine CAS# 134678-17-4 $C_8H_{11}N_3O_3S$ logP: −1.10		99.7	NRTI	112.3 (8)	52.2 (76)	95.3 (44)	Severe and fatal liver problems, high blood acid levels (lactic acidosis), skin reaction
Venlafaxine CAS# 93413-69-5 $C_{17}H_{27}NO_2$ logP: 2.74		100	Selective serotonin reuptake inhibitor (SSNRI) antidepressant	58.2 (16)	77.3 (72)	51.2 (132)	Skin reaction, difficulty breathing
Carbamazepine CAS# 298-46-4 $C_{15}H_{12}N_2O$ logP: 2.77		99.5	Anticonvulsant and mood stabilizer	165.4 (52)	191.5 (56)	62.2 (152)	Ataxia (loss of control of body movement), dizziness, drowsiness
Testosterone CAS# 58-22-0 $C_{19}H_{28}O_2$ logP: 3.37		99	Natural androgen	79.2 (20)	77.2 (52)	51.2 (192)	Endocrine disruptor
Etilefrine (β-blocker) CAS# 709-55-7 $C_{10}H_{15}NO_2$ logP: 0.23		100	Cardiac stimulant	164.5 (8)	77.2 (56)	65.2 (56)	Anxiety, tremors, headaches
Methocarbamol CAS# 532-03-6 $C_{11}H_{15}NO_5$ logP: 0.45			Muscle relaxant	118.3 (8)	199.6 (4)	77.3 (52)	Syncope (temporary loss of consciousness), anaphylaxis, hives, Abdominal pain
Bromacil CAS# 314-40-9 $C_9H_{13}BrN_2O_2$ logP: 2.51		99	Herbicide	54.2 (36)	205.4 (4)	52.2 (40)	Poisoning and toxicity
D7 deethylatrazine CAS # 1216649-31-8		96	Internal standard	195.6 (4)	147.5 (16)	68.2 (48)	n/a

NNRTI = Non-Nucleoside Reverse Transcription Inhibitor (Antiretroviral drug).
NRTI = Nucleoside Reverse Transcription Inhibitor (Antiretroviral drug).
[a] Information available on https://www.drugs.com.

2.3.7. Evaluation of method performance

All native and internal standards were made up to a 1 mg mL^{-1} stock solution in methanol with gravimetric correction for standards with <100% purity. The LC-MS/MS analytical method robustness was successfully tested for precision and repeatability (n = 6 samples, SI Table S4). A combination of standard addition and labelled internal standard calibration was used to calculate recoveries and compensate for matrix effects. Water sample recoveries ranged from 73 to 112% for underivatised compounds and 58 to 87% for derivatised compounds (SI Table S4). Sediment sample recoveries ranged from 84 to 123% for underivatised compounds and 69 to 86% for derivatised compounds (SI Table S4). Limit of detection (LOD) and limit of quantification (LOQ) were calculated at 3× and 10× the signal to noise ratio, respectively. 17-β estradiol, 17-α estradiol, benzestrol and *p*-chloroaniline

Table 2
Derivatised (dansylated) target database and internal standards.

Compound	Structure	Purity (%)	Compound type	Dansylated derivative Mw	Dansylated derivative Mf	Dansylated derivative transition (Collision Energy)	Confirmation ion 1 (Collision Energy)	Confirmation ion 2 (Collision Energy)
17-β-Estradiol CAS# 50-28-2 $C_{18}H_{24}O_2$ logP: 3.75		100	Natural steroid hormone	505.6	$C_{30}H_{35}NO_4S$	506.2 → 171.6 (56)	156.3 (64)	
D4 17-β-Estradiol $C_{18}HD_4H_{20}O_2$ logP: 3.75		99	Internal standard	509.1	$C_{30}H_{39}NO_4S$	510.3 → 171.5 (36)	156.3 (60)	
D4 Estrone CAS#53-16-7 $C_{18}D_4H_{18}O_2$ logP:		99	Internal standard	507.2	$C_{30}D_4H_{29}NO_4S$	508.2 → 171.5 (36)	156.4 (68)	
17-α estradiol 58-22-0 logP 3.75		99	Natural steroid hormone	505.6	$C_{30}H_{35}NO_4S$	506.2 → 171.6 (36)	154.4 (68)	115.3 (100)
Benzestrol CAS# 85-95-0 $C_{20}H_{26}O_2$ logP: 6.10		98	Non-steroidal synthetic estrogen	764.9	$C_{32}H_{37}NO_4S$	765.3 → 281.5 (44)	156.3, (62)	171.6 (58)
p-Chloroaniline CAS# 106-47-8 $ClC_6H_4NH_2$ logP: 1.75		98	Antimicrobial and pesticide and dye precursor	362.1	$ClC_{18}H_{17}N_2O_2S$	363.1 → 168.4 (48)	115.3 (72)	156.5 (44)

Mw = molecular weight.
Mf = molecular formula.

had poor ionization in both positive and negative ionization which resulted in high detection limits. A derivatisation method with dansyl chloride was utilized to lower their limit of detection from ranges of 10–0.125 ng L^{-1} to 0.1–0.04 ng L^{-1} in positive ionization mode. The use of D4 17- β estradiol internal standard was validated against a D4 estrone internal standard to prove that there are no interferences. 17-α estradiol, 17-β estradiol and D4 17- β estradiol were validated and proved to be free from interference. 17-β estradiol and 17-α estradiol peaks were completely resolved by retention time, eluting at 15.488 min and 15.048 min respectively. 17-β estradiol and D4 17-β estradiol coeluted at 15.488 min but were resolved by optimising the dansylated transitions 506.2 → 171.6 and 510.3 → 171.5 respectively.

3. Results and discussion

3.1. Hartbeespoort Dam water pollutants

Efavirenz, nevirapine and carbamazepine were the only EPs found in all water samples in the Hartbeespoort Dam samples, with concentrations generally in the order efavirenz > nevirapine > carbamazepine (Table 3). Methocarbamol, bromacil and venlafaxine could be quantified in 81, 76 and 62% of the water samples, respectively. Steroid hormones analysed could not be detected in any of the water samples tested. On analysis of the surface water ∑11 EP concentrations, the Crocodile River and Magalies River points had the highest and lowest ∑11 EP concentrations respectively in all four seasons (Fig. 3). The other two surface water points of Dam Wall 30 m and Harbour showed trends of varying and fluctuating levels between the four seasons. Unlike herbicides, which are abundant in the summer rainy season, EPs were present in the lowest concentrations in summer which may be attributed to dilution by the high seasonal summer rainfall. Seasonal pollutant concentrations which were in the order spring > winter > autumn > summer were influenced by the rainy seasons as the spring and winter seasons receive the least rainfall.

The most abundant pollutants found in the groundwater were nevirapine and carbamazepine, followed by efavirenz (Table 3). As humans depend on the groundwater as a source of drinking water this exposure pathway may lead to adverse health effects. Nevirapine concentrations in groundwater showed fairly constant levels of 8, 10, 13 and 13 ng L^{-1} for summer, autumn, winter and spring respectively with a relative standard deviation of only 2% for the 4 seasons sampled. Groundwater ∑11EP concentrations were lower than surface water concentrations in three of the four seasons, except spring where the groundwater and Magalies River points recorded ∑11EP concentrations of 29 and 27 ng L^{-1} respectively. Generally, the Magalies River is the least EP impacted surface water point in the Hartbeespoort Dam as it is not located downstream of any major point source, with its water source being the pristine Magalies mountains. The Magalies River passes through rich agricultural lands before pouring into the Hartbeespoort Dam.

There were no significant differences between the Hartbeespoort Dam 5 m and 30 m in EP concentrations in spring. The Crocodile River point which recorded the highest ∑11EP concentrations has the greatest influence on the Hartbeespoort Dam pollution particularly as it has significantly higher flows and contributes 90% of the water in the Hartbeespoort Dam (Amdany et al., 2014).

3.2. Jukskei River water pollutants

In the Jukskei River, the Midrand point, which is affected by multiple point source contamination from raw sewage, recorded the highest ∑EP concentration of 593 ng L^{-1} (Table 3). This is more than twice as much as the Crocodile River winter concentration of 213 ng L^{-1}. The rampant raw sewage contamination may be the primary source of high emerging pollutant levels detected at the Midrand site. The furthermost upstream point of Marlboro recorded the second highest

Table 3
Emerging pollutants determined in the Hartbeespoort Dam catchment and Umgeni River estuary water samples (ng L^{-1}), the Umgeni River estuary sediment (ng g^{-1} dry weight) and Hartbeespoort Dam fish muscle samples (ng g^{-1} fresh weight).

Sample	Nevirapine	Efavirenz	Lamivudine	Emtricitabine	Tenofovir disoproxil	Carbamazepine	Methocarbamol	Etilefrine HCL	Venlafaxine HCL	Bromacil	*p*-Chloroaniline	∑EP site
LOD X3	0.2	0.09	0.05	0.04	0.06	0.04	0.05	0.05	0.06	0.2	0.04	
LOQ X10	0.67	0.3	0.15	0.13	0.2	0.13	0.15	0.15	0.2	0.67	0.13	
Summer												
Croc. R.	35	20	N.D	N.D	N.D	37	7	N.D	1	2	<0.13	102
Dam wall 30 m	25	10	N.D	N.D	N.D	19	6	N.D	N.D	4	N.D	64
Harbour	17	35	N.D	N.D	N.D	27	5	N.D	0.3	3	N.D	87
Magalies R.	6	8	N.D	N.D	N.D	4	1	N.D	<0.2	<0.67	N.D	19
Groundwater	8	3	N.D	N.D	N.D	6	N.D	N.D	N.D	N.D	N.D	17
Autumn												
Croc. R.	42	27	N.D	N.D	N.D	46	20	N.D	3	13	<0.13	151
Dam wall 30 m	40	17	N.D	N.D	N.D	38	15	N.D	2	7	N.D	119
Harbour	39	17	N.D	N.D	N.D	41	12	N.D	1	7	N.D	117
Magalies R.	30	6	N.D	N.D	N.D	28	8	N.D	0.3	5	N.D	77
Groundwater	10	5	N.D	N.D	N.D	8	N.D	N.D	N.D	N.D	N.D	23
Winter												
Croc. R.	44	80	N.D	N.D	N.D	45	34	N.D	3	7	<0.13	213
Dam wall 30 m	39	55	N.D	N.D	N.D	39	33	N.D	1	6	N.D	173
Harbour	43	54	N.D	N.D	N.D	44	28	N.D	2	7	N.D	177
Magalies R.	35	3	N.D	N.D	N.D	23	13	N.D	0.4	5	N.D	79
Groundwater	13	2	N.D	N.D	N.D	11	<0.15	N.D	N.D	N.D	N.D	26
Spring												
Croc. R.	71	303	N.D	N.D	N.D	94	96	N.D	10	3	<0.13	577
Dam Wall 5 m	31	32	N.D	N.D	N.D	33	24	N.D	1	5	N.D	126
Dam Wall 30 m	37	62	N.D	N.D	N.D	31	31	N.D	N.D	4	N.D	165
Harbour	35	82	N.D	N.D	N.D	35	20	N.D	1	5	N.D	178
Magalies R.	6	12	N.D	N.D	N.D	5	3	N.D	<0.2	1	N.D	27
Groundwater	13	3	N.D	N.D	N.D	13	<0.15	N.D	N.D	N.D	N.D	29
Jukskei River winter												
N14	23	134	N.D	0.5	N.D	4	0.4	N.D	<0.2	2	<0.13	164
Kyalami	18	167	N.D	N.D	N.D	17	6	N.D	2	16	N.D	226
Midrand	45	354	N.D	N.D	N.D	75	86	N.D	26	7	<0.13	593
Buccleuch	57	168	N.D	2	N.D	16	48	N.D	3	9	<0.13	303
Marlboro	N.D	302	N.D	13	N.D	18	22	N.D	4	9	<0.13	368
uMngeni River water winter												
Composite	68	138	<0.15	8	N.D	94	3	N.D	1	10	0.76	323
uMngeni River sediment winter			(ng g^{-1} dw)									
U1	81.0	N.D	0.4	1.0	0.2	2.0	0.6	4.0	0.3	8.0	5	103
U2	11.0	N.D	0.6	0.6	0.3	3.0	0.4	4.0	0.8	9.0	0.4	30
U3	N.D	3.0	<0.15	<0.13	<0.2	<0.13	<0.15	<0.15	<0.2	<0.67	0.2	3
U4	N.D	2.0	<0.15	<0.13	<0.2	<0.13	<0.15	<0.15	<0.2	<0.67	0.2	2
Hartbeespoort Dam fish muscle			(ng g^{-1} fw)									
Catfish	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<0.15	<0.2	1	N.D	1
Carp	N.D	N.D	<0.15	N.D	N.D	N.D	N.D	N.D	N.D	<0.67	N.D	0

N.D = not detected.

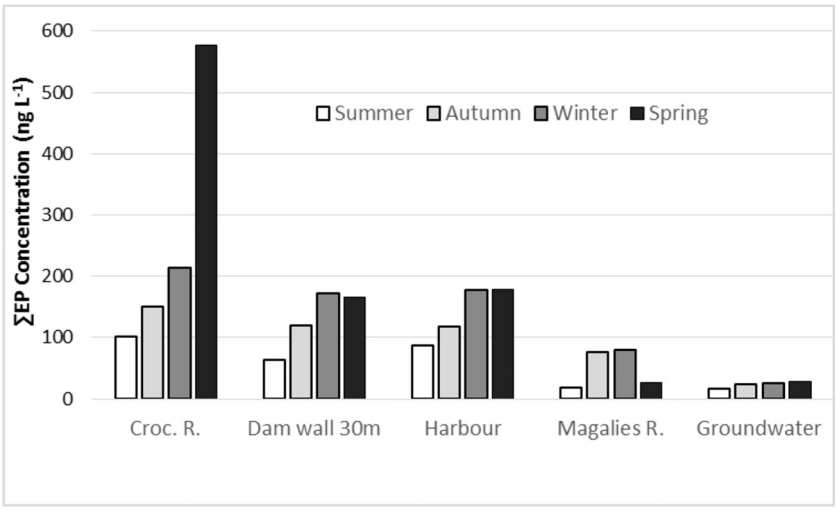


Fig. 3. Seasonal ∑EP concentrations in the Hartbeespoort Dam.

∑11EP concentration of 368 ng L^{-1}. The Marlboro site is downstream of the Alexandra Township whose municipal sewage facilities are overloaded with up to 5 times the volume it was originally designed for. This situation therefore results in frequent unabated raw sewage overflows into the Jukskei River. The Marlboro site is also located downstream of riverside informal settlements such as the Stjwela community in Alexandra which is located right on the bank of the Jukskei River with no municipal sewage facilities.

The Kyalami point located immediately downstream of the Midrand point recorded a significantly lower ∑11EP concentration of 226 ng L^{-1} compared to the upstream Midrand point. Despite being located downstream of the Northern WWTW, the furthermost upstream Jukskei River point of N14 recorded the lowest ∑11EP concentration of 164 ng L^{-1}. This may be due to the dilution effect as the Jukskei River is joined by three tributaries (Fig. 1) before the Northern WWTW. The Northern WWTW does not contribute polar organic pollutants as significantly as the raw sewage contaminating the Jukskei River at Alexandra, upstream of the Marlboro point as well as the informal settlements upstream of the Midrand point. The stench of raw sewage was quite strong in most of the Jukskei River sampling points. Fluctuations in the ∑11EP concentrations along the Jukskei River may be attributed to temporal pollution fluxes due to raw sewage overflows that are rampant along the Jukskei River, particularly upstream of the Northern WWTW.

Jukskei River ∑11EP concentrations were in the order Midrand > Marlboro > Buccleuch > Kyalami > N14. Further downstream of the N14 point, the Crocodile River point had the highest contribution of emerging pollutants into the Hartbeespoort Dam, particularly highest for the spring and winter with ∑11EP concentrations of 577 and 213 ng L^{-1} respectively, indicating that the Jukskei River contributes the greatest emerging pollutant concentrations towards the Hartbeespoort Dam. The Jukskei River between the Midrand, Kyalami and Marlboro has historically been described as similar to sewage in the dry season sampled (Wimberly and Coleman, 1993) and to date, still has the same characteristics. Efavirenz was found in the highest concentrations in all Jukskei River points with significantly higher concentrations in all points compared to the Hartbeespoort Dam points averaging, 6-fold higher in the winter season. Emtricitabine was only detected in the N14, Marlboro and Buccleuch sites throughout the Hartbeespoort Dam catchment with low concentrations of 0.5, 13 and 2 ng L^{-1} respectively (Table 3). *P*-Chloroaniline was detected at very low concentrations, below the LOQ in all the Jukskei River points with the exception of the Kyalami site which had no detectable *p*-chloroaniline. Steroid hormones could not be detected in all Jukskei River water samples and fish muscle tested. In the fish muscle tested, only bromacil could be detected above the LOQ with a concentration of 1 ng g^{-1} in catfish (Table 3), indicating that bioaccumulation of polar emerging compounds in fish muscle is negligible.

3.3. uMngeni River water pollutants

In the uMngeni River composite water sample, efavirenz was found in the highest concentration of 138 ng L^{-1} (Table 3). Carbamazepine and nevirapine were also detected at high concentrations of 94 and 68 ng L^{-1}. Other pollutants were identified at lower concentrations (Table 3) and the steroid hormones analysed could not be detected in the uMngeni water samples. Comparing the ∑11EP water concentrations of the uMngeni River estuary and Jukskei River points, the uMngeni River estuary ∑11EP concentration across the U2 point which recorded 323 ng L^{-1} was only lower than the Midrand and Marlboro points which recorded high values of 593 and 368 ng L^{-1}. The downstream Buccleuch point in the Jukskei River with a ∑11EP concentration of 303 ng L^{-1} is considered a polluted water body where swimming and bathing is prohibited. Based on the concentrations of pollutants detected, re-opening the Umgeni beach to the public after 24 h may have been too hasty, particularly as the cleanup operation was still underway and bottles containing a variety of pharmaceuticals, soaked with water inside were still in the water as well as on the estuary islands and the bank of the uMngeni River estuary.

3.4. uMngeni River sediment pollutants

Nevirapine was found at the highest concentration of 81 ng g^{-1} at U1 (700 m upstream of the uMngeni River mouth) sediments and 11 ng g^{-1} at the U2 site sediments (250 m upstream of the uMngeni River mouth, Fig. 2). Only efavirenz and *p*-chloroaniline could be detected near the sandy estuary mouth (U3 and U4 sites) whilst all other compounds tested were detected at the upstream sites (Fig. 2). Organic pollutants experience dynamic sorption and desorption between the water phase and organic material in sediment. Sediments with high organic carbon content have a greater pollutant sorption capacity. Finer sediment particles consist of higher clay content which have higher organic pollutant sorption characteristics compared to larger sand particles. The sediment composition therefore plays an important role in retention and sorption of organic pollutants. The trends observed may be attributed to the significantly lower TOC and the more sandy samples at U3 and U4 as indicated by the high proportion of sandy particles (2000–425 μm, Fig. 4) particularly at U4 site with no particles at all <100 μm. U1 had the highest proportion of clay particles (<100) μm and U4 had the and highest proportion of sandy particles

(2000–425 μm) respectively. This sediment composition may explain the higher detection rates of the pollutants at U1. Carbamazepine has previously been detected in the uMngeni River catchment sediments with concentrations ranging from 1 to 2.3 ng g^{-1} (Matongo et al., 2015). U1 recorded a similar concentration of 2 ng g^{-1} and U2 recorded a higher concentration of 3 ng g^{-1}. Steroid hormones could not be detected in all the uMngeni estuary sediment samples tested.

4. Limitations of the study

This study is limited to a single sampling regime, meaning that it can be affected by temporal fluxes in API concentrations within the river channels and dam. Though the continuous and steady contamination of the Jukskei River with raw sewage and wastewater treatment plant effluent is not expected to cause major fluxes in API's, this can be addressed through use of a high sampling frequencies or the use of passive samplers to calculate time weighted averages. Useful EP degradation and stability studies throughout the sample collection and extraction stages were not carried out to determine the stability of each compound during sample handling. Development of methodology tailored for diverse emerging pollutants in water and sediments could assist to analyse a wider range of emerging pollutants such as acidic polar organic compounds. Development of enanitoselective methods for profiling chiral APIs which can interact differently with biological organisms, exhibiting different pharmacokinetics is gaining interest in the scientific community and is recommended in future studies.

5. Conclusions

It is proposed that, along with the antibiotics of common usage, the emerging contaminant candidate list should include nevirapine, efavirenz, carbamazepine, methocarbamol, venlafaxine (hydrochloride) and bromacil. The authors suggest for effluent dominated waters, a contaminant candidate list be published by regulatory authorities and either operational or investigative monitoring be conducted using LC-MS/MS. The presence of emerging contaminants in the Hartbeespoort Lake is a cause of major concern for the ecosystem and particularly for fish health and survival as some of the EPs have been proven to cause diminished predator evasive behavior. The Hartbeespoort Dam is a valuable source of fish for the surrounding community and the water is primarily used for irrigating crops, hence the presence of EPs may have an effect on the quality and grade of food. The Crocodile River exerts the most significant impact on the Hartbeespoort Dam pollution, particularly in the dry winter and spring seasons. Further investigation may be conducted for polar emerging pollutants not detected in water by grab sampling by using passive samplers such as polar organic chemical integrative sampler (POCIS) or Chemcatcher.

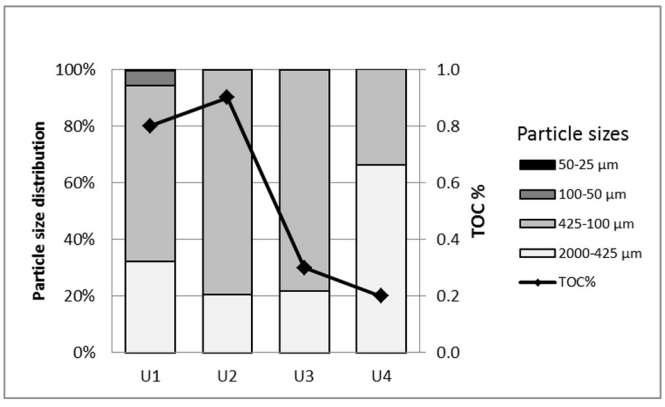


Fig. 4. uMngeni River sediment TOC and particle size distribution.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.263.

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